

CLAIMS:

1. A composition comprising at least two antibodies and a buffer selected from the group consisting of:

- 5 a. 1.5 to 3.0 g/l sodium phosphate monobasic, monohydrate; 0.5 to 0.6 g/l potassium phosphate dibasic, trihydrate; 0.5 to 1.5 ml/l polyoxyethylenesorbitan monolaurate, 50 to 100 ml/l of 5% bovine serum albumin, 0.5 to 1.5 g/l sodium azide, 0.005 g/l, and water and which is at a pH of from about 5.5 to about 6.5;
- 10 b. 40 to 60 ml/l 10X PBS, 0.75 to 1.25 g/l sodium azide, 0.1 to 0.3 ml/l polyoxyethylenesorbitan monolaurate, 45 to 65 g/l bovine serum albumin, and water and which is at a pH of about 7.0 to about 6.5;
- c. 3.0 to 4.0 g/l Tris-hydrochloride, 0.75 to 1.2 g/l sodium azide, 7.5 to 12.5 g/l bovine serum albumin, 0.2 to 0.3 ml/l 25 % hydrochloric acid, and water and which is at a pH of about 5.7 to about 6.5;
- 15 d. one part of a first solution comprising 75 to 125 ml/l 10 X PBS, 2.5 ml/l preservative, 0.25 to 0.75 ml/l of 50% polyoxyethylenesorbitan monolaurate, 2.5 to 7 g/l purified casein, 2.0 to 3.0 ml/l purified Type A gelatin, and water, and a second solution comprising 2.0 to 3.0 g/l sodium phosphate monobasic, monohydrate; 0.5 to 0.6 g/l potassium phosphate dibasic, trihydrate; 0.75 to 1.25 ml/l
- 20 polyoxyethylenesorbitan monolaurate, 75 to 125 ml/l of 2.5 to 7.5 % bovine serum albumin, 0.7 to 1.1 g/l sodium azide, and water, and which is at a pH of about 5.75 to about 6.25; and
- 25 e. 1.5 to 3.0 g/l sodium phosphate monobasic, monohydrate; 0.5 to 0.6 g/l potassium phosphate dibasic, trihydrate; 0.5 to 1.5 ml/l polyoxyethylenesorbitan monolaurate, 50 to 100 ml/l of 5% bovine serum albumin, 0.5 to 1.5 g/l sodium azide,

0.005 g/l, 40 to 60% glycerol, and water, and which is at a pH is about 5.5 to about 6.5.

2. The composition of Claim 1, wherein the buffer is a.

3. The composition of Claim 2, wherein the buffer comprises 2.4g/l sodium phosphate

5 monobasic, monohydrate; 0.56g/l potassium phosphate dibasic, trihydrate; 1 ml/l polyoxyethylenesorbitan monolaurate, 100 ml/l of 5% bovine serum albumin, 0.9 g/l sodium azide, and water and which is at a pH of about 6.0.

4. The composition of Claim 1, wherein the buffer is b.

5. The composition of Claim 4, wherein the buffer comprises 50 ml/l 10X PBS, 0.9
10 g/l sodium azide, 0.2 ml/l polyoxyethylenesorbitan monolaurate, 55 g/l bovine serum albumin, and water, and which is at a pH of about 7.3.

6. The composition of Claim 1, wherein the buffer is c.

7. The composition of Claim 6, wherein the buffer comprises 3.5 g/l Tris-HCl, 0.9 g/l sodium azide, 10 g/l bovine serum albumin, 0.25 ml/l 25 % hydrochloric acid, and water, and
15 which is at a pH of about 6.2.

8. The composition of Claim 1, wherein the buffer is d.

9. The composition of Claim 8, which is at a pH of about 6.0, and wherein the first solution comprises 100 ml/l 10 X PBS, 2.5 ml/l preservative, 0.5 ml/l of 50% polyoxyethylenesorbitan monolaurate, 5 g/l purified casein, 2.5 ml/l purified Type A gelatin,
20 and water; and the second solution comprises 2.4g/l sodium phosphate monobasic, monohydrate; 0.56g/l potassium phosphate dibasic, trihydrate; 1 ml/l polyoxyethylenesorbitan monolaurate, 100 ml/l of 5% bovine serum albumin, 0.9 g/l sodium azide, and water.

10. The composition of Claim 1, wherein the buffer is e.

11. The composition of Claim 10, wherein the buffer comprises 2.4g/l sodium phosphate monobasic, monohydrate; 0.56g/l potassium phosphate dibasic, trihydrate; 1 ml/l polyoxyethylenesorbitan monolaurate, 100 ml/l of 5% bovine serum albumin, 0.9 g/l sodium azide, 50% glycerol, and water, wherein the pH is about 6.0.

5 12. The composition of Claim 1, wherein at least one antibody is from a different species than at least a second antibody.

13. The composition of Claim 1, wherein at least one antibody is a rabbit antibody.

14. The composition of Claim 1, wherein the at least one rabbit antibody is a rabbit monoclonal antibody.

10 15. A method of detecting at least two antigens in a sample, comprising contacting the sample with the composition of Claim 1; and detecting the formation of at least two antigen-antibody complexes formed on the sample.

16. The method of Claim 15, wherein the sample is attached to a solid support.

15 17. The method of Claim 16, wherein the solid support is a glass slide, glass dish, plastic dish, glass well, or plastic well.

18. The method of Claim 16, which further comprises prior to contacting the sample, treating the sample at a temperature of from 65 to 80°C for a time sufficient to adhere the sample to the solid support.

19. The method of Claim 16, wherein the temperature is about 75°C.

20 20. The method of Claim 15, wherein the method is conducted on an automated staining device.

21. The method of Claim 15, wherein at least one antibody is a rabbit antibody.

22. The method of Claim 21, wherein the at least one rabbit antibody is a rabbit monoclonal antibody.

23. The method of Claim 15, wherein detecting the formation of at least two antigen-antibody complexes comprises contacting the sample with a second composition, which comprises at least two antibodies, which specifically bind to the at least two antibodies in the composition wherein at least one antibody in the second composition is coupled to a poly
5 (horseradish peroxidase) and at least a second antibody in the second composition is coupled to a poly (alkaline phosphatase) and wherein the second composition comprises 0.8 to 1.2 M Tris-hydrochloride, pH from 7.3 to 7.9, 0.025 to 0.075% polyoxyethylenesorbitan monolaurate and 2.5 to 3.5 % goat serum; and visualizing at least two antigen-antibody complexes on the sample.

10 24. The method of Claim 23, wherein the second composition further comprises from 3.0 to 3.7 ml/l tris-buffered saline.

25. The method of Claim 23, wherein the second composition further comprises from 3.4 ml/l tris-buffered saline.

15 26. The method of Claim 23, wherein the second composition comprises 0.1M Tris-hydrochloride, pH 7.6, 0.05% polyoxyethylenesorbitan monolaurate, and 3% goat serum.

27. The method of Claim 23, wherein visualizing at least two antigen-antibody complexes on the sample comprising adding at least one chromogen to the sample.

28. A kit comprising the antibody composition of Claim 1 and one or more reagents to detect an antibody-antigen complex.

20 29. The kit of Claim 28, wherein the buffer is a and comprises 2.4g/l sodium phosphate monobasic, monohydrate; 0.56g/l potassium phosphate dibasic, trihydrate; 1 ml/l polyoxyethylenesorbitan monolaurate, 100 ml/l of 5% bovine serum albumin, 0.9 g/l sodium azide, and water and which is at a pH of about 6.0.

30. The kit of Claim 28, wherein the buffer is b and comprises 50 ml/l 10X PBS, 0.9 g/l sodium azide, 0.2 ml/l polyoxyethylenesorbitan monolaurate, 55 g/l bovine serum albumin, and water, and which is at a pH of about 7.3.

31. The kit of Claim 28, wherein the buffer is c and comprises 3.5 g/l Tris-HCl, 0.9 g/l sodium azide, 10 g/l bovine serum albumin, 0.25 ml/l 25 % hydrochloric acid, and water, and which is at a pH of about 6.2.

32. The kit of Claim 28, wherein the buffer is d and which is at a pH of about 6.0, and wherein the first solution comprises 100 ml/l 10 X PBS, 2.5 ml/l preservative, 0.5 ml/l of 50% polyoxyethylenesorbitan monolaurate, 5 g/l purified casein, 2.5 ml/l purified Type A gelatin, and water; and the second solution comprises 2.4g/l sodium phosphate monobasic, monohydrate; 0.56g/l potassium phosphate dibasic, trihydrate; 1 ml/l polyoxyethylenesorbitan monolaurate, 100 ml/l of 5% bovine serum albumin, 0.9 g/l sodium azide, and water.

33. The kit of Claim 28, wherein the buffer is e and comprises 2.4g/l sodium phosphate monobasic, monohydrate; 0.56g/l potassium phosphate dibasic, trihydrate; 1 ml/l polyoxyethylenesorbitan monolaurate, 100 ml/l of 5% bovine serum albumin, 0.9 g/l sodium azide, 50% glycerol, and water, wherein the pH is about 6.0.

34. The kit of Claim 28, wherein at least one antibody is a rabbit antibody.

35. The kit of Claim 34, wherein the at least one rabbit antibody is a rabbit monoclonal antibody.

36. The kit of Claim 28, which further comprises a second composition comprising at least two antibodies wherein at least one antibody is coupled to a poly (horseradish peroxidase) and at least a second antibody is coupled to a poly(alkaline phosphatase) and a buffer comprising 0.8 to 1.2 M Tris-hydrochloride, pH from 7.3 to 7.9, 0.025 to 0.075% polyoxyethylenesorbitan monolaurate and 2.5 to 3.5 % goat serum.

37. The kit of Claim 36, wherein the second composition further comprises from 3.0 to 3.7 ml/l tris-buffered saline.

38. The kit of Claim 36, wherein the second composition further comprises from 3.4 ml/l tris-buffered saline.

5 39. The kit of Claim 36, wherein the buffer in the second composition comprises 0.1M Tris-hydrochloride, pH 7.6, 0.05% polyoxyethylenesorbitan monolaurate, and 3% goat serum.

40. A composition comprising at least two antibodies wherein at least one antibody is coupled to a poly (horseradish peroxidase) and at least a second antibody is coupled to a
10 poly(alkaline phosphatase) and a buffer comprising 0.8 to 1.2 M Tris-hydrochloride, pH from 7.3 to 7.9, 0.025 to 0.075% polyoxyethylenesorbitan monolaurate and 2.5 to 3.5 % goat serum.

41. The composition of Claim 40, which further comprises from 3.0 to 3.7 ml/l tris-buffered saline.

15 42. The composition of Claim 40, which further comprises from 3.4 ml/l tris-buffered saline.

43. The composition of Claim 40, wherein the buffer comprises 0.1M Tris-hydrochloride, pH 7.6, 0.05% polyoxyethylenesorbitan monolaurate, and 3% goat serum.

20 44. The composition of Claim 43, which further comprises from 3.0 to 3.7 ml/l tris-buffered saline.

45. The composition of Claim 40, which further comprises 3.4 ml/l tris-buffered saline.

46. A method of detecting at least two antigens in a sample, comprising contacting the sample with at least one first antibody that binds to a first antigen and at least one second
25 antibody that binds to a second antigen; contacting the sample with the composition of Claim

40, wherein at least one antibody in the composition is binds specifically to the first antibody and another antibody in the composition binds to the second antibody; and detecting the formation of at least two antigen-antibody complexes formed in the sample.

47. The method of Claim 46, wherein the buffer in the composition further comprises
5 from 3.0 to 3.7 ml/l tris-buffered saline.

48. The method of Claim 46, wherein the buffer further comprises 3.4 ml/l tris-buffered saline.

49. The method of Claim 46, wherein the buffer comprises 0.1M Tris-hydrochloride, pH 7.6, 0.05% polyoxyethylenesorbitan monolaurate, and 3% goat serum.

10 50. The method of Claim 46, wherein the method is conducted on an automated staining device.

51. The method of Claim 46, wherein one of the first or second antibodies is a rabbit antibody.

52. The method of Claim 52, wherein the rabbit antibody is a rabbit monoclonal
15 antibody.

53. The method of Claim 46, wherein the sample is attached to a solid support.

54. The method of Claim 53, wherein the solid support is a glass slide, glass dish, plastic dish, glass well, or plastic well.

55. The method of Claim 53, wherein prior to contacting the sample with the first and
20 second antibody, the method comprises treating the sample at a temperature of from 65 to 80°C for a time sufficient to adhere the sample to the solid support.

56. The method of Claim 55, wherein the temperature is about 75°C.

57. A kit comprising the antibody composition of Claim 40, and one or more reagents to detect an antibody-antigen complex.

58. The kit of Claim 57, wherein the buffer further comprises from 3.0 to 3.7 ml/l tris-buffered saline.

59. The kit of Claim 58, wherein the buffer further comprises from 3.4 ml/l tris-buffered saline.

5 60. The kit of Claim 58, wherein the buffer comprises 0.1M Tris-hydrochloride, pH 7.6, 0.05% polyoxyethylenesorbitan monolaurate, and 3% goat serum.

61. A method of detecting at least two antigens in a sample, comprising

contacting a sample with a first antibody;

denaturing the sample with a composition comprising 1 part of a first solution

10 which comprises 1.1 to 1.3 % hydrochloric acid, 0.020 to 0.030 % preservative, and water; and 3 parts of a second solution comprising 0.1 to 0.3 %

polyoxyethylenesorbitan monolaurate, about 0.2 to 0.3 % preservative, and water;

contacting the sample with a second antibody; and

visualizing the formation of at least two antigen-antibody complexes on the

15 sample.

62. The method of Claim 61, wherein the first solution comprises 1.2 % hydrochloric acid, 0.025 % preservative, and water.

63. The method of Claim 61, wherein the second solution comprises 0.2% polyoxyethylenesorbitan monolaurate, 0.25% preservative, and water.

20 64. The method of Claim 61, wherein the sample is attached to a solid support.

65. The method of Claim 64, wherein the solid support is a glass slide, glass dish, plastic dish, glass well, or plastic well.

66. The method of Claim 64, which prior to contacting the sample with the first antibody, the method further comprises treating the sample at a temperature of from 65 to
25 80°C for a time sufficient to adhere the sample to the solid support.

67. The method of Claim 66, wherein the temperature is about 75°C.

68. The method of Claim 61, wherein the method is conducted on an automated staining device.

69. The method of Claim 61, wherein at least one antibody is a rabbit antibody.

70. The method of Claim 69, wherein the at least one rabbit antibody is a rabbit monoclonal antibody.

71. A method of detecting at least two antigens in a sample, comprising contacting the sample with a composition comprising at least one first primary antibody and at least one second primary antibody; and detecting the formation of at least two antigen-antibody complexes on the sample, wherein the at least one first and second antibodies specifically bind to two antigens selected from the group consisting of:

CD3 and Caspase-3;

CD20 and CD3;

CD31 and Ki-67;

CD34 and Factor XIII subunit a;

CDX2 and CK7;

Ki-67 and Caspase-3;

M30 and Ki-67;

LCA and S-100;

CD20 and Ki-67;

Tyrosinase and S100;

Tyrosinase and MART-1;

Tyrosinase and A103;

P63 and CK5;

P63 and P504S;

P63 and P504S;

CK5/6 and Calretinin;

Estrogen receptor and Ki-67;

CK5 and CK17;

5 CD10 and Prostate specific antigen;

CD10 and Hepatic specific antigen;

Chromogranin A and Synaptophysin;

HMW CK and LMW CK;

CD20 and Caspase-3;

10 CD3 and Ki-67;

PAX-5 and CD5;

CD4 and CD8;

Kappa light chain and lambda light chain.

15 72. The method of Claim 71, which comprises detecting at least three antigens in a sample and wherein the method comprises contacting the sample, after detecting the formation of the antigen-antibody complex, with at least a third primary antibody; and detecting the formation of at least third antibody-antigen complex on the sample.

73. The method of Claim 72, which is performed on an automated staining device.

20 74. The method of Claim 71, which comprises detecting at least four antigens in a sample and wherein the method comprises contacting the sample, after detecting the formation of the antigen-antibody complexes, with at least a third and a fourth primary antibody; and detecting the formation of at least third and fourth antibody-antigen complexes on the sample.

75. The method of Claim 74, wherein one of the third or fourth primary antibodies is a rabbit antibody.

76. The method of Claim 75, wherein one of the third or fourth primary antibodies is a rabbit monoclonal antibody.

5 77. The method of Claim 74, which is performed on an automated staining device.

78. The method of Claim 71, wherein one of the first or second primary antibodies is a rabbit antibody.

79. The method of Claim 78, wherein one of the first or second primary antibodies is a rabbit monoclonal antibody.

10 80. The method of Claim 71, which is performed on an automated staining device.

81. A method of detecting two or more antigens in a sample, comprising

contacting a sample, which has been previously contacted with a primary antibody cocktail comprising at least one first primary antibody and at least one second primary antibody, with a composition comprising at least one first secondary antibody and at least one second secondary antibody, wherein the at least one first secondary antibody is coupled to a poly (alkaline phosphatase) moiety and the at least one second secondary antibody is coupled to a poly (horseradish peroxidase) moiety, and wherein the composition comprises a buffer suitable to stabilize the first and second secondary antibodies; and

15

detecting the formation of at least two antigen-antibody complexes on the sample.

20 82. The method of Claim 81, which is performed on an automated staining device.

83. The method of Claim 81, wherein at least three antigens are detected in the sample and the method further comprises contacting the sample, after detecting the formation of the at least two antigen-antibody complexes on the sample, with at least a third primary antibody; and detecting the formation of at least a third antibody-antigen complex on the sample.

25 84. The method of Claim 83, which is performed on an automated staining device.

85. The method of Claim 81, which comprises detecting at least four antigens in a sample and wherein the method comprises contacting the sample, after detecting the formation of the antigen-antibody complexes, with at least a third and a fourth primary antibody; and detecting the formation of at least third and fourth antibody-antigen complexes
5 on the sample.

86. The method of Claim 85, which is performed on an automated staining device.

87. A method of detecting two or more antigens in a sample, comprising
contacting a sample with a primary antibody cocktail comprising at least one first primary antibody and at least one second primary antibody, and subsequently
10 contacting the sample with a composition comprising at least one first secondary antibody and at least one second secondary antibody, wherein the at least one first secondary antibody is coupled to a poly (alkaline phosphatase) moiety and the at least one second secondary antibody is coupled to a poly (horseradish peroxidase) moiety, and wherein the composition comprises a buffer suitable to stabilize the first and second secondary antibodies;
15 and

detecting the formation of at least two antigen-antibody complexes on the sample.

88. The method of Claim 87, which is performed on an automated staining device.

89. The method of Claim 87, wherein at least three antigens are detected in the sample and the method further comprises contacting the sample, after detecting the formation of the
20 at least two antigen-antibody complexes on the sample, with at least a third primary antibody; and detecting the formation of at least a third antibody-antigen complex on the sample.

90. The method of Claim 89, which is performed on an automated staining device.

91. The method of Claim 87, which comprises detecting at least four antigens in a sample and wherein the method comprises contacting the sample, after detecting the
25 formation of the antigen-antibody complexes, with at least a third and a fourth primary

antibody; and detecting the formation of at least third and fourth antibody-antigen complexes on the sample.

92. The method of Claim 91, which is performed on an automated staining device.